A study on the genetic noxiousness of trinitrotoluene

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Abstract In this paper, the mutagencity of TNT was studied for mice and fruit flies. It was indicated that TNT had a great deal of mutagenic effect on the somatic cells and sexual cells of the animals used as the subjects of the experiment, and calculated that TNT would have a genetic harmfulness to human beings.
Key words: Trinitrotoluene, Mouse, Fruit fly, Chromosomal aberration, Sex linkage recessive lethal

Introduction

Trinitrotoluene (TNT), an important material to synthesize gunpower, is one of the common and ancient industry poisons and has a harmfulness to many organs and system such as the liver, blood system, and crystalline lens contacted with it (Althouse 1983). But the reports studying on the chronic genetic noxionsness of TNT are relatively rare so far. Therefore, it is very necessary to study deeply on the mutagenic effects of TNT and to calculate the genetic harmfulness of TNT to various animals (Draggan 1978). In this paper, we have studied that the mutagencity of TNT on the somatic cells and sexual cells, and then probed into the genetic noxiousness of TNT. This has provided a scientific basis for studying on the long term genetic effect of TNT and appraising the safety of TNT.

Materials and methods

Kunming strain of the mice were taken from the Animal Feed Room, Department of Biology, Wuhan University.

Fruit flies (*Drosophila melanogaster)* were taken from the Genetics Laboratory, Department of Biology, Qufu Normal University.

The experiment in the chromosomal aberration used with the mice's bone marrow cell (Wu 1983; Wang 1990) was done in two groups in the routine methods. One is acute group, and another is subacute group. Put 6 mice whose body's weight is 20-25 g in a group according to the sexual proportion (number of the female mice/number of the male mice× 100%). The mice's stomach was poured down with the mixed solution containing TNT every 24 h once, which was compounded the TNT with the maize oil. The treated dosages of TNT in mixed solution in each group was determined by the probationary experiment, respectively 1/6 L_{D50}; 1/20 L_{D50}; 1/60 L_{D50} (The volume of TNT L_{D50} determined by the experiments was equal to 1230.24 mg/kg body's weight). It kept for 2 d in the acute group, but 5 d in the subacute groups. The treatments were done two times in a row again. Besides of it, the mice's stomach were poured down with the maize oil in the negative contrasting groups and these mice's abdominal cavity were injected into with the cyclophosphamide in 30 mg/kg body's weight in the positive contrasting groups. Then we injected the colchine solution into all the mice's abdominal cavity in dosage 4 mg/kg body weight at 6 h after the treatments ended, and 2 h later, got the bone marrow cells from the mice to make the smear. These bone marrow cell smear were stained with Giemsa and 600 cells at metaphase in each mouse were observed under the microscope, and the rates of chromosomal aberration and the cell aberration were calculated.

The all experiment results were inspected by t-test.

The experiment in the sex-linkage recessive lethal with the sexual cell of the fruit flies was done by crossing the wild type of male fruit flies with the Basic strain of female fruit flies according to the method of Muller-5.

The medium of fruit flies

A. The experiment groups: Mixed the TNT and the dimethyl sulfoxide called DMSO for short into the treated medium. Notes: (1) The content of TNT determined by the probationary experiments were equal to 54.27 mg/kg and 13.5 mg/kg medium in each experimental group. (2) The end concentration of DMSO is not more than 2%.

B. The negative contrasting groups: Mixed the maize oil with the normal medium and the end concentration was equal to 2%.

C. The positive contrasting groups: Mixed 0.02 M ethylmethane sulfonate (EMS) with 0.2% sucrose

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solution.

The adult male fruit flies, after hungered for 3~4 d, were put in the medium mentioned above for 4 h and 24 h for each experimental group. 60 fruit flies were put into normal medium bottle in each experimental group and the negative contrasting group, but 10 fruit flies in the positive contrasting group. One pair fruit flies (1 ♂ and ♀) were put into each medium bottle.

♀Basc strain of Virgin flies × ♂ fruit flies
(never treated) | (treated)
↓ normal medium 25±℃
The first filial generation (F1)
↓
The second filial generation (F2)

Observed the results and calculated the lethal rates(%) in follow formula.

$$\text{Lethal rate} = \frac{\text{number of lethal tubes}}{\text{number of lethal tubes + non-lethal tubes}} \times 100\%$$

Results and analyses

Effects of TNT on the chromosomal aberrations

In two experimental methods, the rates of cell aberration and chromosomal aberration in each dosage group have an outstanding difference as with that in the negative contrasting group ($P<0.05$), and a close relationship with the treated dosages of TNT. Their relative coefficients (r) in the acute groups were equal to 0.94 and 0.97, the r in subacute groups were equal to 0.95 and 0.98 ($P<0.05$). At the same time, it is indicated that the rates of the cell aberration and the chromosomal aberration for the mice's bone marrow cells were increasing gradually as the pouring dosages of TNT had been adding and had an outstanding dosage reaction relationship (Shown in Table 1 and Table 2).

Table 1. The rates of mice cell aberration (X±SD; ** P<0.01)

Testing subjects	Number of observed cells	Number of aberrant cells	Acute group aberrational rates (%)	Number of aberrant cells	Subacute group aberrational rates (%)
maize oil	600	8	1.33±0.82**	9	1.50±0.55
cyclophosphamide	600	157	26.17±2.16**	-	-
1/60 LD50TNT	600	34	5.67±2.16**	32	5.33±2.73**
1/20 LD50TNT	600	59	9.83±2.64**	64	10.67±2.07**
1/6 LD50TNT	600	81	13.50±3.72**	89	14.83±2.14**

Table 2. The rate of mice chromosomal aberration (* P<0.05 ** P<0.01))

Testing subjects	Acute group			Subacute group		
	Number of observed chromosomes	number of aberrant chromosomes	Rate of chromosomal aberration(%)	Number of observed chromosomes	Number of aberrant chromosomes	Rate of chromosomal aberration(%)
maize oil	28967	8	0.03	23984	9	0.04
cyclophosphamide	23972	1514	6.32**	-	-	-
1/60 L_{D50}TNT	23972	39	0.16**	23981	45	0.19**
1/20 L_{D50}TNT	23971	76	0.32**	23957	89	0.37**
1/6 L_{D50}TNT	23974	121	0.50**	23964	152	0.63**

It was confirmed that the general laws of changes of these treatments was chiefly chromotid aberrations, particularly the chromotid breakages in various kinds of the chromosomal aberration.

Some aberration of the chromosomal number in each experimental group didn't have statistical meaning as with the negative contrasting group ($P>0.05$). It is indicated that the aberration of the chromosomal number was induced by the artificial factors except TNT, probably some chromosomal breakages in two experimental methods were increasing obviously as with the negative contrasting group, but have no dosage reaction relationship. At the same time, it is shown that the chromosomal breakages were worked as a reference target appraising the mutagenic effect of TNT and the chromosomal aberration was not affected by the sex of the mice and the times of treatment.

Effects of TNT on sex-linkage recessive lethal

The male fruit flies's rate of sexual cell aberration have increased in each experimental group, and the rate of sexual cell aberration in the low dosage groups have an outstanding difference as with that in the negative contrasting group ($P<0.05$). Particularly, it was shown that the difference in the high dosage group was extreme outstanding ($P<0.01$) and had a close relationship with the treated dosages of TNT and the relative coefficient was equal to 0.996 ($P<0.05$). The results indicated that there was a clear dosage reaction relationship between the rate of sexual cell aberration and the treated dosages of

TNT.

The rate of sexual cell aberration in three experimental groups were equal to 3.1, 4.5 and 8.2 times as negative contrasting group, and it is shown that TNT have lead to the male fruit flies's sexual cells to happen mutation (Shown in Table 3).

Table 3. The rate of sexual cell mutation (* $P<0.05$ ** $P<0.01$)

Testing subjects	Test1		Test2		Test3		Test4	
	M/C	Mutational rate(%)	M/C	mutational rate(%)	M/C	mutational rate(%)	M/C	mutational rate(%)
negative contrast	1/600	0.33	2/600	0.33	0/598	0	3/1798	0.17
positive contrast	22/97	22.68**	6/90	6.67**	23/94	24.27**	5/281	18.15**
low dosage	3/600	0.50	5/600	0.83	3/599	0.50	11/1799	0.61**
middle dosage	4/599	0.67	7/598	1.17	2/598	0.33	13/1795	0.72**
high dosage	7/600	1.17**	12/597	2.01**	6/595	1.00*	25/1792	1.40*

Note: M/C=Number of mutatant cells/Number of observed cells

There were no outstanding differences among the rates of sexual cell aberration in three experimental groups ($P<0.05$), and results indicated that TNT had induced mutational effects to the sexual cells of the male fruit flies in some phase of cell division and didn't affect the sexual cells in another phases.

Discussion

Experiment on chromosomal aberration

The results of experiment have shown that TNT increased not only the rate of cell aberration rates but also the rate of chromosomal aberration. This is because it got into the cells of these animals and would have existed in cell protoplasm and nucleus after mice stomach were poured by mixed solution TNT or the fruit flies were cultured in the medium contained TNT. When TNT in the cells were reacted with some oxidases, the nitro (NO_3^-) in the molecular would transformed into nitroso (NO_2^-), which is a intense induced mutational agent. If the nitroso should arrived at the DNA molecular it would changed the structure of bases in the DNA molecular and then result would be that one base transformed into another base, called base pair subsititution, or bring about the breakage of single strand DNA (Oppenoorth 1979). This would be why the treated fruit flies had shown more chromotid breakages. Therefore, these results suggested that the mutagenic agent had serious damaging effect to the chromosomes.

In addition to, it was shown that TNT would change a few of the chromosomal number, yet. When cell protoplasm contained TNT it would inhibited the synthesis of some proteins and affected to formation of spindle among phase of cell division, and then chromosomes division would be abnormal (Wang 1993). Certainly, when TNT have integrated with other chemical compound of the cell it would produced a various effects (Chen 1996), which included other different damaging.

Experiment on the sex-linkage recessive lethal

Because TNT would changed the structure of base and lead to the substitution of base pair of DNA molecular it not only can induced mutation of somatic cell and but the mutation of sexual cell also happened, which were obvious in the sex linkeage recessive lethal particularly (Chen 1996). TNT have closely relationship with human beings. TNT may damage the somatic and sexual cells of human beings and caused mutation of sexual cells and a genetic sex linkage recessive lethal and other diseases. TNT has more harmfulness to human beings and other animals. So we must pay attention to prevent the seriously effects of mutagencity and harmfulness of TNT to human beings and other living things.

References

Althouse, R. *et al* compiled, Wang Rukan translated. 1983. Chemical Materials and Cancer in Man. People Hygienical Press. pp.19-22

Chen Guizhu, Miao Shenyu, Huang Yushan *et al.* 1996. Allocation, cycle and purged effects of nutrient pollutant N is artificial wastewater in simulated *Kandelia candel* wetland system. Acta Sci. Circumstantiae. **16**(1): 44-50

Draggan, S and Gidding Jeffery, M. 1978. Testing toxic substances for protection of the environment. Sci. Total Environ., **9**(1): 63-74

Oppenoorth, J.M. 1979. Influence of Cycloheimide and actinomycin D on initiation and early development of adventitious roots. Physiologia. Plantarum, **47**(2): 134-138

Wang Lunshan *et al.* 1993. The influence of actionmycin and cyloheximide on the formation of the masses of embroygenic cells and the globular embroyes and on metabolic kinesis of macromolecule in embryogenic calli of *F. pallidiflora* Schrenk. Acta Genetica Sinica. **20**(2): 159-166

Wang Zongren *et al.* 1990. Experimental Methods of Animal Genetics. Beijing University Press.pp.96-113

Wu Heling *et al.*1983. Methods and Technology on Genetics Experiment. Advanced Education Press.pp.167-169; pp.217-221